iGEM TU/e 2014
Biomedical Engineering

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FACS - DBCO-PEG$_4$-5/6-TAMRA

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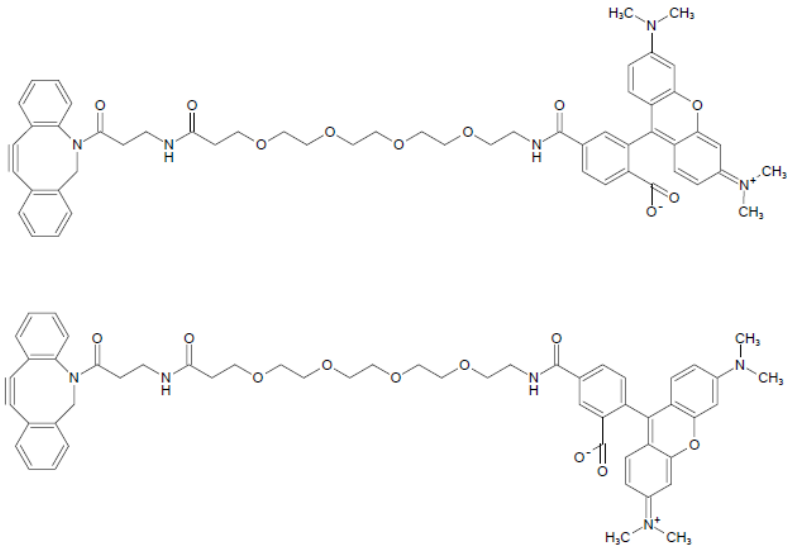
FACS - DBCO-PEG4-5/6-TAMRA

1 Stock solutions



DBCO-PEG$_4$-5/6-TAMRA, molecular weight: 936.06 g/mol; λ_{abs} 545 nm; λ_{em} 565 nm

- DBCO-PEG$_4$-5/6-TAMRA in DMSO (5 mM)
- DBCO-PEG$_4$-5/6-TAMRA in DMSO (833 µM)
- Buffer: PBS

2 Preparation of FACS samples

- Prepare following tubes:

Tube	[DBCO]	Buffer volume to add	Cells (10^9/mL)	DBCO volume to add (µL)		DBCO/tag ratio
				5 mM	833 µM	
1	0	150 µL	50 µL			
2	30 µM	0 µL	200 µL	1.21		1,1E+04
3	5 µM	0 µL	200 µL		1.21	1,8E+03
4	30 µM	100 µL	100 µL	1.21		2,2E+04
5	5 µM	100 µL	100 µL		1.21	3,6E+03
6	30 µM	150 µL	50 µL	1.21		4,4E+04
7	5 µM	150 µL	50 µL		1.21	7,3E+03

- React DBCO tubes for 1h or 6h a in shaking block at 4°C and 500 rpm
- Prepare FACS samples after 1 and 6 hours:
  - Spin down the cells for 5 min at 13,400 rpm
  - Resuspend with 1 mL ice cold PBS
  - Spin down the cells for 5 min at 13,400 rpm and put on ice until FACS
  - Right before FACS: resuspend with 200 µL PBS